Delhi Pharmaceutical Sciences and Research University

Synopsis

For

Doctor of Philosophy (Ph.D.)

Govt. of N.C.T of Delhi,

Pushp Vihar, Sector-III, M.B. Road,

New Delhi-110017

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Delhi Pharmaceutical Sciences and Research University

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Registration No. :11/PhD/DPSRU/2018

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Project Title: Structural investigation into identification of small molecules that can modulate the lipid homeostasis: A computational study

Executive summary of Ph.D. work

A. Title of work

*"Structural investigation into identification of small molecules that can modulate the lipid homeostasis: A computational study"***.**

B. Introduction

The work mainly focuses on the identification of the small molecules through computer and structure guided approaches which can modulate the lipid homeostasis.

C. Literature Review

The prerequisite of the small molecules discovery required thorough understanding of the fundamental and basic principles underlying lipid homeostasis. Therefore, the literature survey was carried out and all the information related to the key receptors and its structure and small molecules are collected to create a database. Secondly to curate the information about the receptors and their structure-function relationship with their binding partners to establish the mechanistic understanding of key proteins and its structural regions which involved in the regulatory process. Among these receptors FXR is expressed in the liver and master regulator of the bile acid synthesis. All receptors have various biological function and there is various ligand reported against it. Here, we are using the vast computational approaches encompassing the chemo-informatics, structural bioinformatics and computational biophysics followed by in vitro validation to identify the small molecule which can modulate the lipid homeostasis.

D. Aim of the Study

The present work involves the structural insights in to identification of the small molecules that can modulate the lipid homeostasis. We are applying the chemo-informatics and computational-biophysical approaches to define structure-activity relationships between the reported compounds and the receptor with the goal of generating rationally designed novel and more potent molecules that interact selectively with the receptor proteins. The manually curated selective drugs (against target protein) database and its exploration at atomic level resolution are paving additional insights in hits selection and hit-to-lead conversion. At last the computational selected molecules will be experimentally validated. The study will include the following objectives.

a) **Objective 1** To understand the structural and functional properties of FXR through molecular modeling and reported experimental studies.

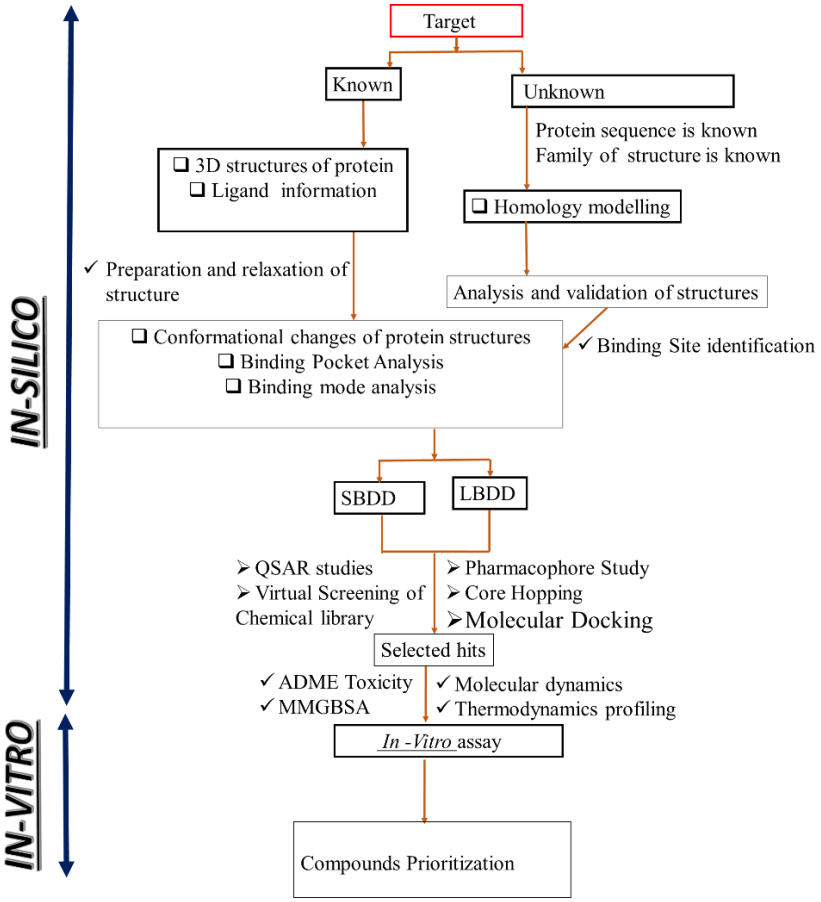
b) **Objective 2** To understand the rational of reported biologically active compounds of FXR agonist.

c) **Objective 3** To explore the protein-protein interaction (PPI) interface to understand the allosteric mechanism of the binding partners.

d) **Objective 4** *In-silico* identification of active compounds and their possible experimental validation

E. Methodology of the work

The pipeline for the work involves the various computational approaches which helps in hit identification followed by experimental validation.



Scheme: Computational work-flow pipeline

Expected Outcomes

The structural and functional insights will guide us to understand the biological mechanism of action of protein. The structure- and ligand- based approaches help us to identify the small molecule which can modulate the lipid homeostasis.

Synopsis for Ph.D. work

Title

"*Structural investigation into identification of small molecules that can modulate the lipid homeostasis: A computational study*"

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1. Background

The lipid homeostasis is the process of maintenance of an internal steady state of lipid within an organism or cell. The maintenance of lipid homeostasis is multi-layered and includes biosynthesis, degradation, transport and transduction network. There are more than 10,000 molecular species of lipids present in the biological membrane and store the large amount of structural and functional information in form of lipids as the data suggested by the lipid metabolome analysis. The primary function of these membrane lipids is to form the permeability barrier of the cell, they are enzymatically converted to the bioactive lipids such as prostaglandin, leukotriene, ceramide, inositol phosphate, playing a crucial role in "Signal transduction network" that control cellular metabolism and organism's activities. Lipid molecules also form a "Transport network of lipids", which distributes lipid molecules to various cellular membranes and tissues. The "Biosynthetic network of lipids" regulated by biochemical mechanisms of the cell cycle to coordinate the extensive degradation and synthesis of lipids during membrane biogenesis as well as synthesis of cellular components such as DNA and proteins is yet unexplored. In addition, some lipid metabolites are key players as fuel sensing and signalling molecules, forming an "Energy production and storage network", which controls energy homeostasis at its cellular and organism level [1].So the maintenance of lipid level is important. The dysfunctioning of the lipid homeostasis involved in the various metabolic disorder such as obesity, type 2 diabetes, hyperlipidaemia, NAFLD, NASH and other infectious and immune related disorder. The work is focus on the liver lipid homeostasis level so that we can identify the molecule to modulate the lipid homeostasis and have therapeutic application in liver related disorders.

2. Introduction

Bile acids are endocrine molecules which contribute to several essential functions including cholesterol catabolism and intestinal lipid emulsification. Liver is the site for the bile acids synthesis and converts cholesterol to bile acids through the classic, alternative and neutral pathways [2,3]. It also plays a central organ for coordinating much of metabolism and has a large capacity for fatty acid uptake[4,5]. Bile acids activates the nuclear receptors (farnesoid X receptor, pregnane X receptor, vitamin D receptor and membrane-bound receptors including G protein coupled receptor (TGR5), sphingosine-1-phosphate receptor 2 (S1PR2) and cholinergic receptor muscarinic 2(CHRM2)[6,7] and various cell signalling pathways (c-Jun N-terminal

kinase 1/2, AKT, and ERK 1/2) which alters the expression of numerous genes encoding enzyme/proteins involved in the regulation of bile acid, glucose, fatty acid, lipoprotein synthesis, metabolism, transport, and energy metabolism. In liver, the bile acids regulates the expression of a number of transport proteins and biosynthetic enzymes which are crucial for the maintenance of BA and lipid homeostasis[8]. These include NTCP, the hepatic basolateral sodium taurocholate cotransporter protein[9]: BSEP, the hepatic canalicular bile salt export pump[10]; and CYP7A a cholesterol 7α-hydroxylase which is the rate-limiting enzyme for the production of BAs from cholesterol via the neutral biosynthetic pathway in liver [11]. Among these receptors FXR is expressed in the liver and master regulator of the bile acid synthesis. All these receptors have various biological function and there is various ligand reported against it. Here, we are using the vast computational approaches encompassing the chemo-informatics, structural bioinformatics and computational biophysics followed by in vitro validation to identify the small molecule which can modulate the lipid homeostasis.

Key Words: Bile acids, Farnesoid X receptor, Cholesterol 7 alpha-hydroxylase.

3.1 FXR family

The farnesoid X receptor (FXR; Gene symbol: NR1H4) belonging to the superfamily of nuclear receptors (NRs)[12] one of the 48 ligand-activated nuclear transcription factor proteins and highly expressed in the liver and intestine. FXR plays a crucial role in hepatic triglyceride (TG) homeostasis, as well as in glucose metabolism [13]. In addition, FXR lowers hepatic TG content and serum TG levels and improves insulin resistance and hyperglycaemia. Therefore, the modulation of FXR receptor became an attractive area in drug discovery to develop novel therapeutic opportunities for liver and metabolic disorders.

3.2 Mechanisms of ligand binding to FXR

The ligand binding to FXR regulates a large number of target genes involved in bile acid, lipoprotein and glucose metabolisms. FXR exerts its function by eliciting transcriptional alterations [15]. It binds to DNA either as a heterodimer with RXR or as a monomer to regulate the expression of various genes. In common with other NRs, the FXR protein exhibits a modular structure, and contain several autonomous functional domains: an N-terminal region with a ligand-independent activation function (AF1), a highly conserved zinc-finger DNA-binding domain (DBD) that is connected to the ligand binding domain (LBD) by a flexible

hinge region [16]. The LBD contains two well-conserved regions: a signature motif and the AF2 motif located at the carboxy-terminal end of the domain which is responsible for ligand-dependent transactivation function [17]. Upon agonist binding to FXR creates the huge conformational rearrangement due to which the dissociation of co-repressors and the recruitment of co-activators occur to promote the transcriptional initiation. Understanding of conformational changes at the binding of co-repressor/activator is also not explored much at molecular level.

3.3 Availability of crystal structures of target proteins

In FXR we have curated the ~ 82 PDB structures reported to the PDB bank (repository has been created) **Figure (1)**. We study the LBD domain of the FXR protein to discover the potent modulator against it. The FXR protein consist of the 12 helical in its LBD domain. The crystal structure of LBD domain is shown in **Figure (2A)**. During the study we found that the binding of ligands with FXR involves the steroidal and nonsteroidal modulator to activate the functioning of the FXR[18].The FXR LBD complexed with agonist show a canonical transcriptionally active conformation where helix H12 folds back against the LBD core as shown in **Figure (2B)** , the LBD to entrap the ligand (‘mousetrap’ model) and contributes to the formation of the coactivator binding groove [19]. Therefore, stabilization of H12 in this position is probably due to direct contacts between ligand and helix, and/or via ligand-dependent stabilization of contacts between the helix and the LBD core. So, first aim of study to dig out the structural information of the FXR and the conformational changes occur in FXR upon binding of ligands.

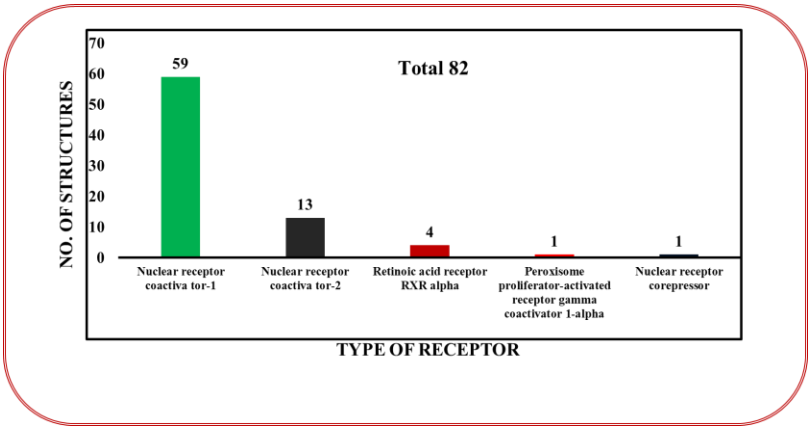

Figure 1: STRUCTURE STATISTICS

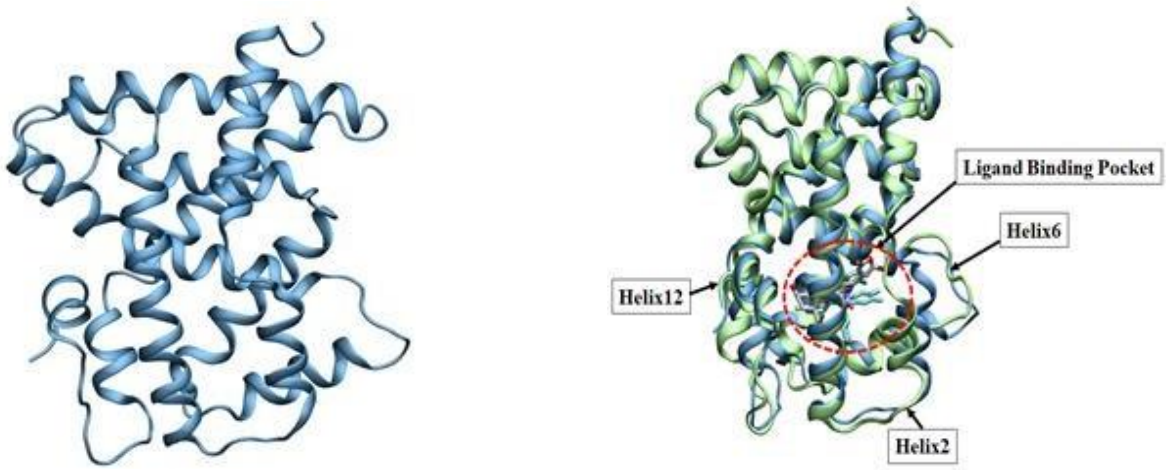

Figure 2A) Structure of FXR**.** **B)** Structural changes in ligand binding.

3.4 Ligands of FXR

Activation of FXR can be achieved using selective ligands which includes the bile acids as the endogenous ligands for FXR, in both conjugated and unconjugated forms [20] and nonsteroidal ligands. To further investigate the structure-activity relation of FXR activation, we found that FXR activated by the synthetic steroidal like OCA are ~100-fold more agonist activity than the endogenous ligands (CDCA) and many of agonists have been tested in clinical trials for treating metabolic disorder [21].There is the certain drug which are either approved or in the developmental phase for FXR as shown in table.

Table: Drugs in market or in clinical trial (https://clinicaltrials.gov/)

Name of Compounds	Primary indications	Development phase
OCA	PBC	Approved
	NASH	Phase III
	PSC	Phase II
	Biliary Atresia	Phase I

INT-767	Hepatic fibrosis	Phase I
Tropifexor (LNJ-452)	PBC	Phase II
	NASH	Phase II
GS-9674 (Px-104)	PBC	Phase II
	PSC	Phase II
	NASH	Phase II
EDP-305	NASH	Phase I
UDCA	PBC	Phase II
LMB763(Nidufexor)	NASH	Phase I

4. Objectives of Study

Objective 1

To understand the structural and functional properties of FXR through molecular modeling and reported experimental studies.

(a) Literature survey to understand the lipid homeostasis.
(b) To understand the architecture of ligand binding domain (LBD) of FXR and activation function 2 (AF2) domain.
(c) To understand the binding pocket of FXR (LBD) in APO states and with their binding partners (corepressor or coactivators).

Objective 2

To understand the rational of reported biologically active compounds of FXR agonist.

(a) To curate the database for FXR at its structural and ligand level.

(b) To study the conformational structural changes in the protein structures in COM (reported most potent steroidal and non-steroidal compounds).

(c) To identify and quantify the energy-contribution per residues-wise in terms of binding affinity and its correlation with experimental values (EC50/IC50/Kd)

(d) To explore the thermodynamic landscape through free energy calculation.

Objective 3

To explore the protein-protein interaction (PPI) interface to understand the allosteric mechanism of the binding partners.

(a) To understand the interfaces of FXR with RXR and co-activator/co-repressor from reported crystal structures.

(b) To apply the different PP docking algorithms to get the consensus results for establishing our docking benchmark.

(c) To understand the conformational changes of the complexes by using the molecular dynamics simulation techniques.

(d) To identify the *hot-spots* regions and key residues from the PPI interfaces.

Objective 4

***In-silico* identification of active compounds and their possible experimental validation**

(a) To identify and filter out the computationally active compounds using curated virtual compounds libraries against FXR and its interfaces from different complexes.

(b) To validate the identified potent compounds into experimental assay.

5. Methodology and techniques to be used during the study

I. Computational Approaches:

a) Retrieval of three dimensional structures from Protein Data Bank (PDB) database

b) Molecular Modelling to generate the complete structure of targets

c) Molecular Docking and Virtual Screening

d) Structure- and ligand-based methods for hit designing

e) Conventional Molecular Dynamics (MD) Simulation and Free energy calculations

f) Advanced computational chemistry for novel series of compound designing

g) Biased molecular dynamics simulations for better understanding of thermodynamic profiling and association-dissociation process.

II virtual screening of the compounds from the available libraries.

III. Experimental validations of the identified potent compounds into experimental assay.

Conclusion

The structural and functional insights will guide us to understand the biological mechanism of action of protein. The structure- and ligand- based approaches help us to identify the small molecule which can modulate the lipid homeostasis.

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